Synthesis, Characterization and Evaluation of Isoniazid Analogues as Potent Anticancer Agents

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Summary

A series of *(E)-N'-(substituted-benzylidene)isonicotinohydrazide* derivatives were synthesized by coupling it with different substituted aldehydes, acetophenones and benzophenones in presence of absolute ethanol along with catalytic amount of glacial acetic acid. The entire synthesized compound were confirmed and characterized by using various spectral technique like IR, ^{1}H NMR and ^{13}C NMR spectroscopy studies. All the synthesized compounds were evaluated for their Anti-cancer activity against Lung, Colon, CNS, Ovarian, Renal and Prostate Cancer. Among all synthesized derivatives, analogue **(2b)** *(E)-N-(4-fluorobenzylidene)isonicotinohydrazide* and **(2p)** *(E)-N-(4-nitrobenzylidene)isonicotinohydrazide* was found to exhibit significant anticancer activity as compare to the standard drug Etopside.

Keywords: Hydrazones, Isoniazid, Lipophilicity, Anticancer activity

Introduction

Cancer is a collective term used for a group of diseases that are characterized by loss of control to grow, divide, and spreading of cells, leading to a primary tumor that invades and destroy other tissues. It may also spread to other regions of the body through a process known as metastasis which is the cause of 90% of cancer deaths. Cancer is normally caused by abnormalities of genetic material of the affected cell. Tumorigenesis is the multistep process that involves the accumulation of successive mutations in oncogenes and suppresser genes that deregulate the cell cycle. Tumorigenic events include small scale change in DNA sequences, such as point mutation, large scale chromosomal aberrations, such as translocations, deletion, and amplifications, and changes that affect the chromatin structure and are associated with dysfunctional epigenetic control such as aberrant methylation of DNA or acetylation of histones[1]. Cancer is not only a cell disease but also a tisular disease in which the normal relationships

between epithelial cells and their underlined stroma cells are lost[2]. Cancer is not a disease but it is a cluster of diseases. Most cancer are named after the organ type of cell in which they start such as cancer that begin in colon is called colon cancer, that begins in the basal cell of the skin is called basal cell carcinoma[3]. Unlike infectious diseases such as AIDS, influenza, tuberculosis, cancer is not contagious[4]. Cancer is actually a group of more than one hundred separate diseases. These diseases are all characterized by an abnormal and unregulated growth of cells. This growth destroys surrounding body tissues and may spread to other parts of the body in a process that is known as metastasis[5]. Cancer can develop in almost any tissue, such as lung, colon, breast, skin, bones or nerve tissue[6].

Consequently, the development of newer anticancer agents will remain an important challenging task for medicinal chemists[7]. So, there is an urgent need for identification of novel lead structure for the designing of new, potent, and less toxic agents which ideally shorten the duration of therapy and are effective against cancer[8]. Hydrazone belong to schiff base family contain azomethine – NHN=CH protons constitute an important class of compounds for new drug development[9]. Day by day, the chemistry of carbon-nitrogen double bond of hydrazone is fast becaming the backbone of condensation reaction in benzo-fused N-Hetrocycles[10]. Many researchers have synthesized these compounds as target structure and evaluated their biological activities. Hydrazones have been reported to possess, antitumoral[11,12], antimicrobial[13], antitubercular[14,15], anticonvulsant[16], analgesic[17], anti-inflammatory[18,19], antiplatelet[20], antifungal[21], antiviral[22], antibacterial[23] and antimalarial activities[24].

Among the important pharmacophores responsible for anticancer activity, the hydrazone scaffold is still considered a viable lead structure for the synthesis of more efficacious and broad spectrum anticancer agents. Further, pharmacokinetic and cellular permeability of the drug can be increased by derivatization to bioreversible form of this drug, namely hydrazone. It is belived that hydrazone functional group increase the lipophilicity of parent amine and amides and results into enhancement of absorption through biomembranes and this enhanced lipophilicity of hydrazones. Inspired by the above facts and in continuation of our on going research program in the field of synthesis and anticancer activity of medicinally important compound it was thought that it would be worthwhile to design, synthesize some new hydrazone derivatives and screen them for anticancer activities.

Material and Methods

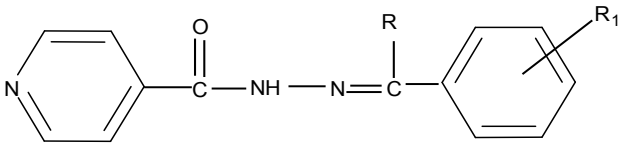
Melting points of the synthesized compounds were determined in open-glass capillaries on Stuart SMP10 melting point apparatus and were uncorrected. The purity of the compounds was checked by thin layer chromatography (TLC). Silica gel plates kiesel gel 0.25 mm, 60 GF_{254}, precoated sheets obtained from Merck, Darmstadt (Germany) were used for TLC and the spots were visualized by ultraviolet light as visualizing agent. The IR spectra (υ, cm^{-1}) were obtained with a Perkin-Elmer 1600 FTIR spectrometer in KBr pellets. ^{1}H-NMR spectra (δ, ppm) were recorded in DMSO-d_6 solutions on a Varian-Mercury 300 MHz spectrometer using tetramethylsilane as the internal reference. ^{13}C NMR spectra were recorded in DMSO-d_6 solutions on a Bruker Avance II 400 spectrometer at 400 MHz using tetramethylsilane as the internal reference. Elemental analyses were performed on an ECS 4010 Elemental Combustion System. The necessary chemicals were purchased from Loba Chemie and Sigma Aldrich.

Synthesis of substituted aryl acid hydrazones derivatives (2a-2v)

The synthesis of the 21 isonicotinohydrazide derivatives, (**2a-2v**), was published elsewhere[25] and performed with good yields from commercially available materials. The synthetic strategies adopted to obtain the target compounds (**2a-2v)** are depicted in Scheme 1. The type of substituted aldehydes, acetophenones and benzophenone are given in table **1**. The equimolar quantities of substituted

aldehydes, acetophenones and benzophenones (50 mmol) were refluxed with isonicotinic acid hydrazide (50 mmol) in absolute ethanol (50 ml) along with catalytic amount of glacial acetic acid. The reaction mixture was refluxed for 5-9 h and the completion of reaction was confirmed by thin layer chromatography. After being cooled and concentrated, the product was added in ice cold water. The precipitate was collected through filtration and dried in oven at low temperature. The crude products were recrystallised from absolute ethanol to give desired products.

Table 1. Detail of different substituted Aldehydes, Acetophenones and Benzophenones



Sr. No.	R	R_1
2a.	H	2-F
2b.	H	4-F
2c.	H	2-I
2d.	H	2,4-NO_2
2e.	H	2-OCH_3
2f.	H	3-OCH_3
2g.	H	4-OCH_3
2h.	H	2-OC_2H_5
2i.	H	3-OC_2H_5
2j.	H	4-OC_3H_7
2k.	H	3,4-OCH_3
2l.	H	3-OCH3, 4-OH
2m.	H	4-OH
2n.	H	4-$N(CH_3)_2$
2o.	H	2-NO_2
2p.	H	4-NO_2
2r.	CH_3	4-OH
2s.	CH_3	3-NH_2
2t.	CH_3	4-NH_2
2u.	C_6H_5	H
2v.	C_6H_5	4-Br

Results and Discussion

The synthetic strategies adopted to obtain the target compounds **2a-2v** are depicted in Scheme **1**. Equimolar quantity of substituted benzaldehydes, acetophenones or benzophenones that reacts with the equimolar quantity of isoniazid in ethanolic medium is refluxed for 7 h to form acid hydrazones. The completion of reaction was confirmed by thin layer chromatography (TLC). The types of substituted aldehydes, acetophenones and benzophenones are given in Table **1**. The purity of the compounds was checked by TLC, elemental analyses and characterized by spectral data. In general, IR spectra of all

compounds showed absorption band at around 3290-3186, 3176-3026, 1691- 1659, 1662-1625, 1583-1548 cm-1 regions, conforming the presence of NH, CH, C=N, C=O, C=C respectively. The ^{1}H NMR spectra, the signals of the respective prepared derivatives were verified on the basis of their chemical shifts, multiplicities, and coupling constants. The spectra of most compounds showed the characteristic NH proton at (δ 12.63-11.52 ppm), 4H proton of pyridine was at around (δ 9.05-7.25 ppm), ^{1}H proton of -N=C-H (δ 8.92-8.30 ppm) and characteristic protons of benzylidene at (δ 8.79-6.75 ppm). ^{13}C-NMR spectra of compounds **2a-2v** characteristic C=O signals appeared at around (δ 163.59-162.92 ppm), benzylidene (δ 168.89-113.36 ppm), -N=C-H (δ 143.72-142.19 ppm), pyridine **(**δ 149.89-121.17 ppm). Data obtained were found to be in good agreement with the calculated values of proposed structure.

Anticancer Activity

Evaluation of anticancer activity was performed on the compounds inconvertibly selected by National Institute of Health, Bethesda, USA under the Drug Discovery Programme of NCI. The human tumor cell lines of the cancer screening panel were grown in RPMI 1640 medium containing 5% fetal bovine serum and 2 mM L-glutamine. For a typical screening experiment, cells were inoculated into 96 well micro titer plates in 100 µL at plating densities ranging from 5,000- 40,000 cells/well depending on the doubling time of individual cell lines. After cell inoculation, the micro titer plates were incubated 37°C, 5% CO_2, 95% air and 100% relative humidity for 24 hour prior to addition of experimental drugs. After 24 h, two plates of each cell line were fixed in situ with TCA, to represent a measurement of cell population for each cell line at the time of drug addition (Tz). Experimental drug was solubilized in dimethyl sulfoxide at 400-fold the desired final maximum test concentration and stored frozen prior to use. At the time of drug addition, an aliquot of frozen concentrate was thawed and diluted to twice the desired final maximum test concentration with complete medium containing 50 µg/mL gentamicin.

The test drug of 5 Molar was added to the cell lines. Aliquots of 100 µl of this different drug dilution were added to the appropriate micro titer wells already containing 100ml of medium. Following drug addition the plates were incubated for an additional 48 hour at 37˚C, 5% CO_2, 95% air, and 100% relative humidity. For adherent cells, the assay was terminated by the addition of cold TCA. Cells were fixed in situ by the gentle addition of 50 µl of cold 50% (w/v) TCA (final concentration, 10% (TCA) and incubated for 60 min at 4˚C. The supernatant was discarded, and the plates were washed five times with tap water and air dried. Sulforhodamine B (SRB) solution (100 µl) at o.4% z (w/v) in 1% acetic acid was added to each well, and plates were incubated for 10 min at room temperature. After staining, unbound die was removed by washing five times with 1% acetic acid and the plates were air dried. Bound stain was subsequently solubilized with 10 mM trizma base, and the absorbance was read on an automated plate reader at a wavelength of 515 nm.

For suspension cells the methodology was the same except that the assay was terminated by fixing settled cells at the bottom of the wells by gently adding 50 µl of 80% TCA (final concentration, 16% TCA). Using the seven absorbance measurements [time zero, (Tz), control growth, (C), and test growth in the presence of the drug at the 1.00E-5 Molar concentration level (Ti)], the percentage growth inhibition was calculated as:

[(Ti-Tz)/(C-Tz)] × 100 for concentration for which Ti>/=Tz

[(Ti-Tz)/Tz] × 100 for concentration for which Ti< Tz

Growth inhibition of 50% (GI50) was calculated from [(Ti-Tz)/(C-Tz)] × 100 = 50, which is the drug concentration in a 50% reduction a net protein increase (as measured by SRB staining) in control cells during the drug incubation. The drug concentration resulting in total growth inhibition (TGI) was calculated from Ti=Tz. The LC50 (concentration of drug resulting in a 50% reduction in measured protein at the end of the drug treatment as compared to that at the beginning) indicating a net loss of cells following treatment was calculated from [(Ti-Tz)/Tz] × 100 = -5.

Values were calculated for each of these three parameters if the value of activity was reached however, if the effect is not reached or was exceeded, the value for that parameter was expressed as greater or less than the maximum or minimum concentration tested. The compounds which reduced the growth of any one of the cell lines by 32% or less are passed on for further evaluation in the full panel of 60 cell lines[26]. Only two compounds have shown the prominent results and their *In-vitro* cytotoxicity reports were shown in Table **2**.

Table 2 *In-vitro* cytotoxicity of the synthesized compounds

Cancer cell lines	**2b**	**2p**
Leukemia		
CCRF-CEM	87.85	85.94
HL-60 (TB)	95.67	100.21
K-526	100.04	98.27
MOLT-4	110.99	88.26
RPMI-8226	104.85	96.34
Non-Small Cell Lung Cancer		
A549/ATCC	100.81	85.38
EKVX	93.49	109.10
HOP-62	87.41	102.20
NCI-H226	103.76	102.26
NCI-H23	87.29	93.95
NCI-H322M	93.65	135.66
NCI-H522	83.01	98.31
Colon Cancer		
COLO 205	113.87	105.11
HCC-2998	98.51	93.58
HCT-116	99.09	91.20
HCT-15	109.45	102.87
HT29	104.34	84.03
KM-12	104.03	100.40
CNS Cancer		
SF-268	90.45	111.62
SF-295	93.88	110.33
SF-539	96.27	87.38
SNB-19	99.06	84.39
SNB-75	86.55	102.96
Cancer cell lines	**2b**	**2h**
Melanoma		
Lox IMVI	104.84	90.57
MALME-3M	101.64	110.51
M-14	97.05	98.37
MDA-MB-435	97.97	106.76
SK-MEL-2	99.53	96.40
SK-MEL-5	102.02	101.84

Ovarian Cancer		
IGROV1	81.62	111.62
OVCAR-3	96.14	116.68
OVCAR-4	101.19	111.31
OVCAR-5	94.53	92.71
OVCAR-8	101.72	101.01
Renal Cancer		
786-0	96.07	88.22
ACHN	105.87	95.64
CAKL-1	90.16	92.82
RXF 393	104.97	121.44
SN12C	105.10	92.22
Prostate Cancer		
PC-3	95.25	97.26
DU-145	112.94	112.42
Breast Cancer		
MCF7	96.4	99.87
MDA-MB-231/ATCC	109.87	95.73
BT-549	100.09	83.55
T-47D	92.41	109.15
MAD-MB-468	117.88	109.19

Value > 100 indicates absence of activity

Conclusions

All the synthesized hydrazone analogues have been evaluated for their Anti-cancer activity against Lung, Colon, CNS, Ovarian, Renal and Prostate Cancer. Among all synthesized derivatives, analogue (**2b)** *(E)-N-(4-fluorobenzylidene)isonicotinohydrazide* and (**2p)** *(E)-N-(4-nitrobenzylidene)isonicotinohydrazide* was found to exhibit significant anticancer activity as compare to the standard drug Etopside. The isonicotinoyl hydrazides synthesized by condensing isoniazid with various aldehydes, acetophenones and benzophenones displayed moderate to potent anticancer activity.

References

1. Nelson SM, Ferguson LR, Denny WA. DNA and the chromosome–varied targets for chemotherapy. Cell Chromo. Rev. Cell Chromosome 2004; 3: 2-17.
2. Bissell MJ, Radisky D. Putting tumours in context. Nat. Rev. Cancer. 2001; 1: 46-54.
3. Macloed K. Oncogenes and cell proliferation. Curr. Opin. Genect. Dev. 2000; 10: 81-93.
4. Kilpatrick SE, Geisinger KR. Soft tissue sarcomas: the usefulness and limitations of fine-needle aspiration biopsy. Am. J. Clin. Pathol. 1998; 110: 50-68.
5. Pagano JS, Blaser M, Buendia, MA. Infectious agent and cancer: criteria for a casual relation. Semin. Cancer. Biol. 2004; 14: 453-471.
6. Brennan MJ, Endocrinology in cancer of the breast. Status and prospects. Am. J of clin. Pathology. 1975; 64: 797-809.
7. Verma A, Saraf SK. 4-thiazolidinone-a biologically active scaffold. Eur J Med Chem. 2008; 43, 897-905.
8. Murphy ST, Case HL, Ellsworth E, Hagen S, Husband M, Jonnides T, Limberakis C, Marotti KR, Ottolini AM, Rauckhorst M, Starr J, Stier M, Taylor C, Zhu T, Blasser A, Denny WA, Lu GL, Smailic

JB, Rivault F. The synthesis and biological evaluation of novel series of nitrile-containing fluoroquinolones as antibacterial agents. Bioorg. Med. Chem. Lett. 2007; 17: 2150-2155.
9. Rollas S, Kucukguzel SG. Biological Activities of Hydrazone Derivatives. Molecule. 2007; 12: 1910-1939.
10. Rashed N, El Massry AM, El Ashry ESH, Amer A, Zimmer H. A facile synthesis of novel triazaloquinoxalinoxalinones and traizinoquinolones. J. Heterocycl. Chem. 1990; 27: 691-693.
11. El-Hawash SAM, Abdel WAE, El-Dewellawy MA. Synthesis of Some New Quinoxalines and 1,2,4-Triazolo[4,3-*a*]-quinoxalines for Evaluation of *in vitro* Antitumor and Antimicrobial Activities. Arch. Pharm. Chem. Life Sci. 2006; 339, 564-571.
12. Cocco MT, Congiu C, Lilliu V, Onnis V. Synthesis and in vitro antitumoral activity of new hydrazinopyrimidine-5-carbonitrile derivatives. Bioorg. Med. Chem. 2005; 14: 366-372.
13. Rollas S, Gulerman N, Edeniz H. Synthesis and Antimicrobial Activity of Acid Hydrazide and 3-Acetyl-2,5-Dis- ubstituted-1,3,4-Oxadiazolines. Farmaco. 2002; 57: 171-174.
14. Imramovsky A, Polanc S, Vinsova J, Kocevar M, Jampitek J, Reckova Z, Kaustova JA. A new modification of anti- tubercular active molecules. Bioorg. Med. Chem. 2007; 15: 2513-2551.
15. Janin, Y. Antituberculosis drugs: Ten years of research. Bioorg. Med. Chem. 2007; 15: 2479-2513.
16. Dimmock JR, Vasishtha SC, Stables JP. Anticonvulsant properties of various acetylhydrazones, aromatic and unsaturated carbonyl compounds. Eur. J. Med. Chem. 2000; 35: 241-248.
17. Lima PC, Lima LM, Silva KC, Leda PH, Miranda ALP, Fraga CAM, Barreiro EJ. Synthesis and analgesic activity of novel *N*-acylarylhydrazones and isosters, derived from natural safrole. Eur. J. Med. Chem. 2000; 35: 187-203.
18. Salgin-Goksen U, Gokham-Keleci N, Gostal O, Koysal Y, Kilici E, Isik S, Aktay G, Ozalp, M. 1-Acylthiosemicarbazides, 1,2,4-triazole-5(4*H*)-thiones, 1,3,4-thiadiazoles and hydrazones containing 5-methyl-2-benzoxazolinones: Synthesis, analgesic-anti-inflammatory and antimicrobial activities. Bioorg. Med. Chem. 2007; 15: 5738-5751.
19. Kalsi R, Shrimali M, Bhalla TN, Barthwal JP. Synthesis and anti-inflammatory activity of indolyl azetidinones. Ind. J. Pharm. Sci. 2006; 52: 129-134.
20. Silva GA, Costa LMM, Brito FCF, Miranda ALP, Barreiro EJ, Fraga CAM. New class of potent antinociceptive and antiplatelet 10*H*-phenothiazine-1-acylhydrazone derivatives. Bioorg. Med. Chem. 2004; 12: 3149-3158.
21. Loncle C, Brunel JM, Vidal N, Dherbomez M, Letourneux Y. Synthesis and antifungal activity of cholesterol-hydrazone derivatives. Eur. J. Med. Chem. 2004; 39: 1067-1071.
22. Abdel-Aal, MT, El-sayed WA, El-ashry EH. Synthesis and antiviral evaluation of some sugar arylglycinoylhydrazones and their oxadiazoline derivatives. Arch. Pharm. Chem. Life Sci. 2006; 339: 656-663.
23. Capilla J, Serena C, Javier F, Ortoneda T, Guarro J. Efficacy of Voriconazole in Treatment of Systemic Scedosporiosis in Neutropenic Mice. Antimicrob. Agents Chemother. 2003; 47: 3976-3978.
24. Walcourt A, Loyevsky M, Lovejoy DB, Gordeuk VR, Richardson DR. Novel aroylhydrazone and thiosemicarbazone iron chelators with anti-malarial activity against chloroquine-resistant and -sensitive parasites. Int. J. Biochem. Cell Biol. 2004; 36: 401-407.
25. Malhotra M, Sharma R, Monga V, Deep A, Sahu, Kapendra; Samad, Abdul. Synthesis, Characterization of (E)-N'-(substituted-benzylidene)isonicotinohydrazide Derivatives as Potent Antitubercular Agents. 2011; 8: 575-579.
26. Karali N. Synthesis and primary cytotoxicity evaluation of new 5-nitroindole-2.3-dione derivatives. Eur. J. Med. Chem. 2002; 37, 909-918.